ACTION OF IONS ON THE HEART OF THE OYSTER (*OSTREA VIRGINICA*)

EDWARD M. WALZL

DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

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ACTION OF IONS ON THE HEART OF THE OYSTER (OSTREA VIRGINICA)[1]

(Ten figures)

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RINGER (1882) showed that sodium, potassium, and calcium salts are normally present in definite proportions in the blood of vertebrates, and are necessary for the proper functioning of the tissues. He studied the effect of these salts mainly on the heart of the frog, and found that excess of sodium or potassium in the fluid perfusing the heart causes cessation of beating in a relaxed condition, i.e., arrest in diastole, while excess of calcium causes cessation of beating in a contracted condition, i.e., arrest in systole; also that deficiency of sodium or potassium has the same effect as excess of calcium, while deficiency of calcium has the same effect as excess of either of the other two.

Experiments on the effects of salts have been performed on the hearts of a large number of other species of animals by other investigators. The results obtained on the hearts of all vertebrates are in general agreement with those obtained by Ringer on the heart of the frog, but the results obtained on the hearts of a considerable number of invertebrates often appear to be the reverse in kind of those obtained with vertebrate hearts.

The results obtained with the hearts of invertebrates show that excess of NaCl causes arrest in systole in *Limulus* (Carlson, 1906), *Maia* and *Homarus* (Hogben, 1925), *Cambarus clarkii* (Lindeman, 1928), and *Helix pomatia* (Cardot and Jullien, 1930); that excess of KCl causes arrest in systole in *Aplysia* (Heymans, 1923) and *Helix* (Cardot and Jullien, 1930); and that excess of $CaCl_2$ causes arrest in diastole in *Aplysia* (Heymans, 1923), *Helix* (Cardot, 1921, and Hogben, 1925), *Ostrea circumpicta* Pils. (Takatsuki, 1927), and *Maia* (Hogben, 1925); but that excess of either potassium or calcium causes arrest in systole in *Pecten* (Mines, 1912) and *Anodon* (Koch, 1916).

[1] I wish to express my appreciation to Professor S. O. Mast, under whose direction this problem was carried out, for his many suggestions, especially in reference to the preparation of this paper for publication; and to Professor Ivon R. Taylor, Brown University, for his help and supervision of all phases of the work.

I am also greatly indebted to the Biological Laboratory, Cold Spring Harbor, L.I., N.Y., for a grant which covered nearly all the expenses incurred, and for providing excellent laboratory facilities, apparatus, etc.

A thorough investigation of the action of salts on one of the invertebrates exhibiting anomalous effects is obviously necessary in attempting to explain the differences observed. The heart of the oyster exhibits anomalous effects to a marked degree, and, since it is readily obtained and kept, it was chosen for the following experiments.

METHODS

Large oysters of uniform size were collected in Oyster Bay, Long Island Sound, during July and kept in a live box in Cold Spring Harbor until used. All experiments were made during July, August, and September.

The heart of the oyster is located in the pericardial cavity directly behind the adductor muscle. The rectum runs adjacent to the tip of the ventricle, and is closely attached to the arteries which leave the ventricle and run anteriorly and posteriorly, respectively, along the gut, but it does not penetrate the substance of the heart as is true of some lamellibranches. The walls of the auricles are thin, and they are composed of unstriated muscle fibers which are loosely interwoven and present a meshlike appearance.

The excised heart of the oyster, like that of other marine invertebrates, beats normally for from several hours to several days if it is perfused with sea water or van't Hoff solution. However, since the oysters used were collected in brackish waters, and freezing-point determinations of the blood of these oysters showed it to be hypotonic with van't Hoff solution, this solution was diluted until its conductivity equaled that of the blood of the oyster. The concentration of the salts in the diluted van't Hoff solution follows: 0.36 molar NaCl, 0.028 molar $MgCl_2$, 0.014 molar $MgSO_4$, 0.008 molar KCl, 0.011 molar $CaCl_2$.

The solutions used to ascertain the effect of different ions on the heart consisted either of solutions of single salts or of diluted van't Hoff solution in which the relative concentrations of the salts had been changed. Dextrose was added to the solutions of single salts, if necessary, to make them isosmotic with the blood of the oyster. The solutions were unbuffered, but acid or base was added so that, immediately before using, the H-ion activity was the same as that of the blood of the oyster. The solutions were protected from access of carbon dioxide by means of soda-lime tubes, and tests showed no appreciable changes in the H-ion activity during the experiments. Mallinckrodt's or Baker's Analyzed salts were used. Solutions of deliquescent chlorides were standardized against silver nitrate according to the Volhard-Drechsel method. A triple-tandem pyrex glass still containing (*a*) $KMnO_4$, (*b*) $Ba(OH)_2$, and (*c*) distilled water (Mast, 1928) was used in preparing the distilled water for the solutions. The solutions were prepared and mixed immediately before using.

The hearts used were prepared for perfusion as follows: The shell was opened by cutting the adductor muscle, and the heart canulated by inserting a small glass canula, through a cut in one of the auricles, into the ventricle (Fig. 1). It was then tied in place by a ligature at the auriculo-ventricular junction, after which the heart was removed and the canula connected to the outlet of the stopcock in the perfusion chamber (Fig. 1). Tracings of the contractions of the heart were made on a long-paper kymograph by means of a writing lever.

In testing the action on the heart of solutions, they were put into bottles *A2*, *A3*, and *A4*, and van't Hoff solution into *A1* (Fig. 2). The stopcocks *S1–S4* (Fig. 2) were then adjusted until the liquid rose to the same level in the manometers *M1–M4*, so as to make the flowing pressure during perfusion the same for all solutions. The perfusion fluid was

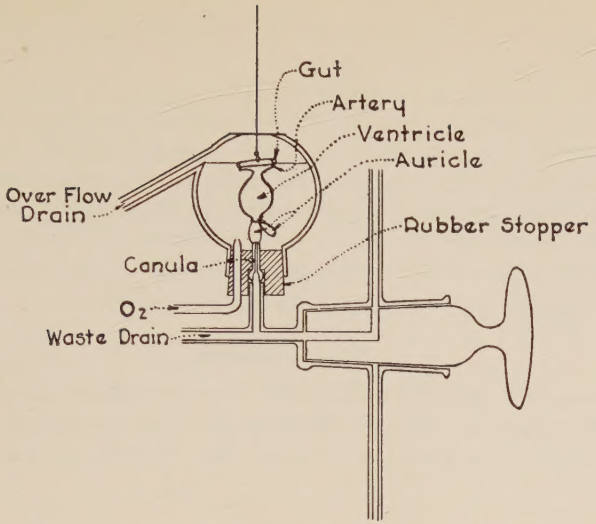


FIG. 1.—Sketch showing method of perfusion of the heart

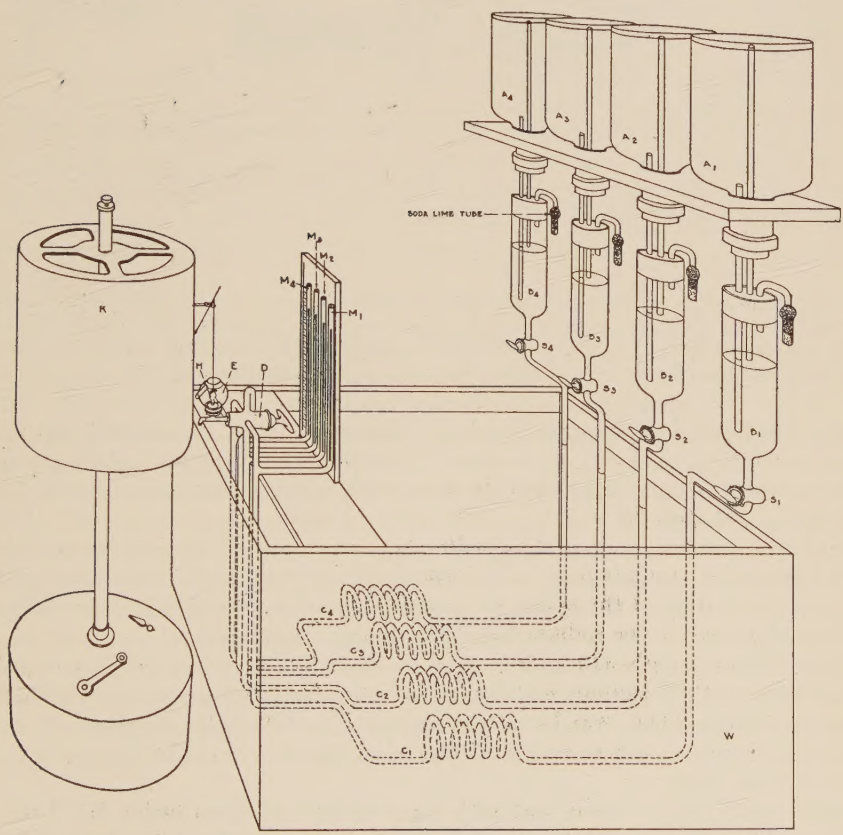


FIG. 2.—Sketch showing arrangement of apparatus used in perfusing the heart of the oyster. *A1–A4*, reservoirs containing perfusion fluids; *B1–B4*, constant-level tubes; *D*, four-way stopcock; *H*, heart; *K*, kymograph; *M1–M4*, manometers; *S1–S4*, stopcocks for regulating the flowing pressures; *V*, perfusion chamber; *W*, constant-temperature water bath.

brought to 18° C. by passing it through thin-walled pyrex-glass coils, *C1–C4* (Fig. 2) in a thermostatically controlled water bath. Tests showed that the temperature of the fluid in the heart chamber did not vary by more than 0°.5 C.

The observations concerning the effects of salts were made as follows: The excised heart was perfused with van't Hoff solution until the beat had become well established; then it was perfused with the solution to be tested until the character of the response again became steady; after which it was again perfused with van't Hoff solution. If the heart did not completely recover, the results were discarded.

SODIUM CHLORIDE

The action of sodium chloride on the hearts of several other mollusks closely related to the oyster, *O. virginica*, has been studied by other investigators and extremely diverse results were obtained. Heymans (1923) found that solutions of NaCl cause arrest in

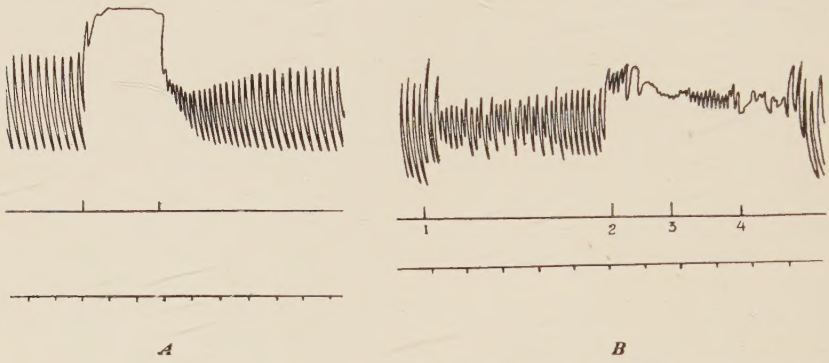


FIG. 3.—Graphs showing the effect of solutions containing different concentrations of NaCl, made isotonic with the blood of the oyster by the addition of dextrose. *A*, 0.418 molar; *B:* (1) van't Hoff solution changed to 0.104 molar NaCl, (2) 0.104 molar NaCl changed to 0.418 molar NaCl, (3) 0.418 molar NaCl changed to 0.104 molar NaCl, (4) 0.104 molar NaCl changed to van't Hoff solution.

systole in the heart of the parapod, *Aplysia;* Takatsuki (1927) maintained that it exerts little or no effect on the heart of the oyster, *O. circumpicta* Pils.; and Cardot and Jullien (1930) concluded that sodium chloride specifically inhibits contraction in the heart of the land snail, *H. pomatia.*

It has been maintained that the results obtained from perfusion with pure solutions of NaCl are due to the absence of the other necessary salts, and consequently do not give a true indication of the action of sodium. However, if various concentrations are used, the effect due to the sodium ion should become apparent.

Solutions containing 0.418 molar, 0.209 molar, and 0.04 molar NaCl and sufficient dextrose to make them isotonic with the blood of the oyster were tested on a considerable number of hearts. There was but little variation in the results obtained. Those of a typical experiment on one heart are presented in Figure 3, and the average for all the experiments in Table 1.

Figure 3 and Table 1 show that with 0.418 molar and 0.209 molar NaCl the heart immediately contracted and remained in this condition without beating until it was again perfused with van't Hoff solution; and that the greater contraction was obtained

with the stronger solutions. Figure 3*b* and Table 1 show that with 0.104 molar NaCl the heart did not stop beating, but that the extent of relaxation decreased; and that, if the heart is perfused with 0.104 molar NaCl after it has stopped beating in 0.418 molar NaCl, it resumes beating. This demonstrates clearly that the stoppage of the heart with 0.418 molar NaCl is due to action of the sodium ion and not to lack of the other ions.

TABLE 1

EFFECT ON THE BEAT OF THE HEART OF THE OYSTER OF SOLUTIONS OF DIFFERENT CONCENTRATIONS OF NaCl, MADE ISOTONIC WITH THE BLOOD OF THE OYSTER BY THE ADDITION OF DEXTROSE

Concentration of NaCl	Percentage Change in Frequency	Percentage Change in Amplitude	Change in Extent of Relaxation (Mm.)
0.418 molar	−100	−100	−7.1
0.209	−100	−100	−3.5
0.104	0	− 27	−1.8

It can be concluded that solutions of sodium chloride inhibit relaxation in the heart of the oyster and, in high concentrations, cause arrest with augmentation of normal contraction. These results are obviously not in accord with the findings of Ringer (1882), Lussana (1911), Pond (1920), and others with vertebrate hearts, or with the conclusions of Cardot and Jullien (1930) that sodium specifically inhibits contraction in the heart of *Helix;* but they are in accord with those of Carlson (1906) on the heart of *Limulus*, and Hogben (1926) on those of *Homarus* and *Maia.*

POTASSIUM CHLORIDE

PURE SOLUTIONS OF KCl IN DIFFERENT CONCENTRATIONS

Experiments similar to those just described were performed on a considerable number of hearts with solutions containing the following concentrations of KCl: 0.418 molar, 0.209 molar, 0.104 molar, 0.04 molar, and 0.008 molar, and sufficient dextrose to make them isotonic with the blood of the oyster.

Figure 4 and Table 2 show that KCl in concentrations from 0.418 molar to 0.04 molar causes the heart to stop in a contracted state which is greater than that of normal systole, but that the extent of contraction varies directly with the concentration of KCl; and that 0.008 molar KCl does not cause the heart to stop immediately but causes decrease in frequency and amplitude and a tendency to sustained contraction.

These results are somewhat similar to those obtained with solutions of NaCl. The relation between concentration of salt and extent of contraction for NaCl and KCl is presented in Figure 5. This figure shows that solutions of KCl produce greater contraction than solutions of NaCl of the same concentration, and that greater concentrations of NaCl are required to stop the heart than of KCl.

EXCESS OF KCl IN VAN'T HOFF SOLUTION

Experiments were performed with van't Hoff solutions with the concentration of KCl increased two, four, and six times, respectively (0.016 molar, 0.032 molar, and 0.048

molar), but with the concentrations of the other salts unchanged. The results of some of these experiments are given in Figure 6*a* and Table 2.

Reference to Figure 6*a* and Table 2 shows that, if the heart is perfused with van't Hoff solution containing excess of KCl, the frequency of beat increases and the extent of relaxation decreases. Comparison of the results obtained with different concentrations of KCl presented in Table 2 shows that, if the concentration of the salt is increased, the extent of relaxation decreases until with the concentration of KCl increased six times the heart stops in systole.

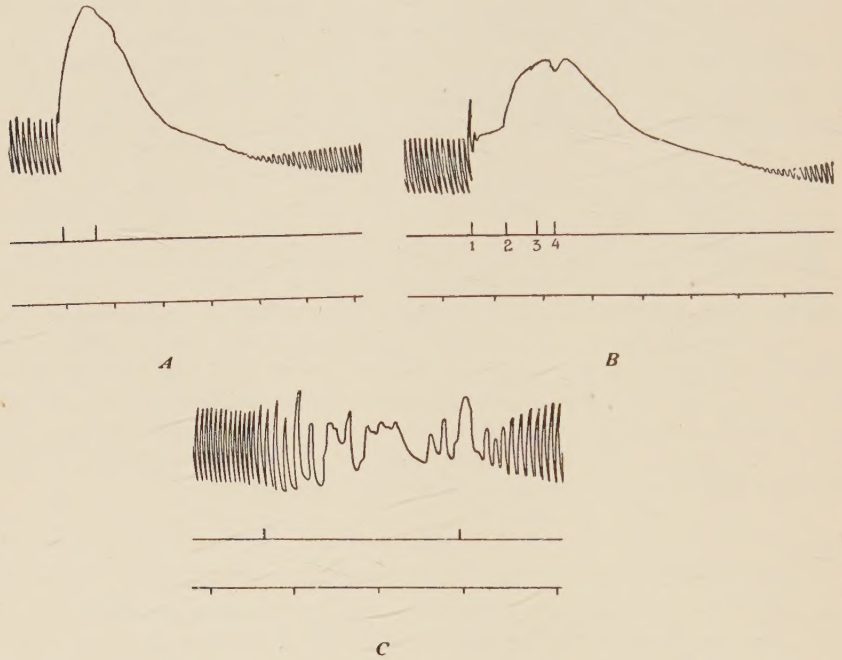


FIG. 4.—Graphs showing the effect of solutions of different concentrations of KCl, made isotonic with the blood of the oyster by the addition of dextrose. *A*, 0.418 molar KCl, *B:* (1) van't Hoff solution changed to 0.04 molar KCl, (2) 0.04 molar KCl changed to 0.209 molar KCl, (3) 0.209 molar KCl changed to 0.418 molar KCl, (4) 0.418 molar KCl changed to van't Hoff solution; *C*, 0.008 molar KCl.

DEFICIENCY OF KCl IN VAN'T HOFF SOLUTION

Experiments were performed in which the concentration of KCl in van't Hoff solution was decreased from 0.008 molar to 0.0045 molar, 0.003 molar, 0.0015 molar, 0.0007 molar, and 0.0 molar, respectively. The results of these experiments are presented in Figure 6*b* and *c* and Table 2.

These results show that decrease in concentration of KCl in van't Hoff solution to 0.003 molar causes increase in frequency—and inhibition of relaxation—but no change in extent of contraction at systole; that decrease to 0.0015 molar causes decrease in frequency and extent of contraction as well as inhibition of relaxation; and that further decrease causes arrest in systole.

It has been frequently assumed in the literature that, if excess of a salt produces a

certain effect, deficiency of it should produce the opposite effect. This obviously does not obtain with the oyster heart. The results obtained indicate that there is an optimum concentration of KCl, and that either increase or decrease in this concentration results in inhibition of relaxation. It is of interest to observe that, whereas excess of KCl causes arrest in diastole in vertebrate hearts and arrest in systole in the oyster heart, deficiency of KCl has the same effect on both, i.e., inhibition of relaxation.

TABLE 2

EFFECT ON THE BEAT OF THE HEART OF THE OYSTER OF SOLUTIONS OF DIFFERENT CONCENTRATIONS OF KCl, MADE ISOTONIC WITH THE BLOOD OF THE OYSTER BY THE ADDITION OF DEXTROSE, AND OF VAN'T HOFF SOLUTIONS WITH DIFFERENT DEGREES OF EXCESS AND DEFICIENCY OF KCl

Concentration of KCl	Percentage Change in Frequency	Percentage Change in Amplitude	Change in Extent of Relaxation (Mm.)
	KCl in Dextrose Solutions		
0.418 molar	−100	−100	−8.5
0.209	−100	−100	−6.8
0.104	−100	−100	−4.6
0.04	−100	−100	−4.1
0.008	− 50	− 53	−0.8
	KCl in van't Hoff Solution (Excess)		
0.016	+ 80	− 82	−3.3
0.032	+ 25	− 99	−4.6
0.048	−100	−100	−4.9
	KCl in van't Hoff Solution (Deficiency)		
0.0045	+ 9	− 47	−1.6
0.003	+ 33	− 73	−2.1
0.0015	− 6	− 83	−1.6
0.0007	−100	−100	−2.8
0.0	−100	−100	−3.8

CALCIUM CHLORIDE

PURE SOLUTIONS OF $CaCl_2$ IN DIFFERENT CONCENTRATIONS

Experiments were made with four different concentrations of $CaCl_2$ (0.418 molar, 0.209 molar, 0.104 molar, and 0.04 molar) on a considerable number of hearts. The results are presented in Figure 7 and Table 3. Figure 7*a* and Table 3 show that 0.418 molar $CaCl_2$ causes the heart to stop beating immediately in a state of relaxation much greater than it is in normal diastole.

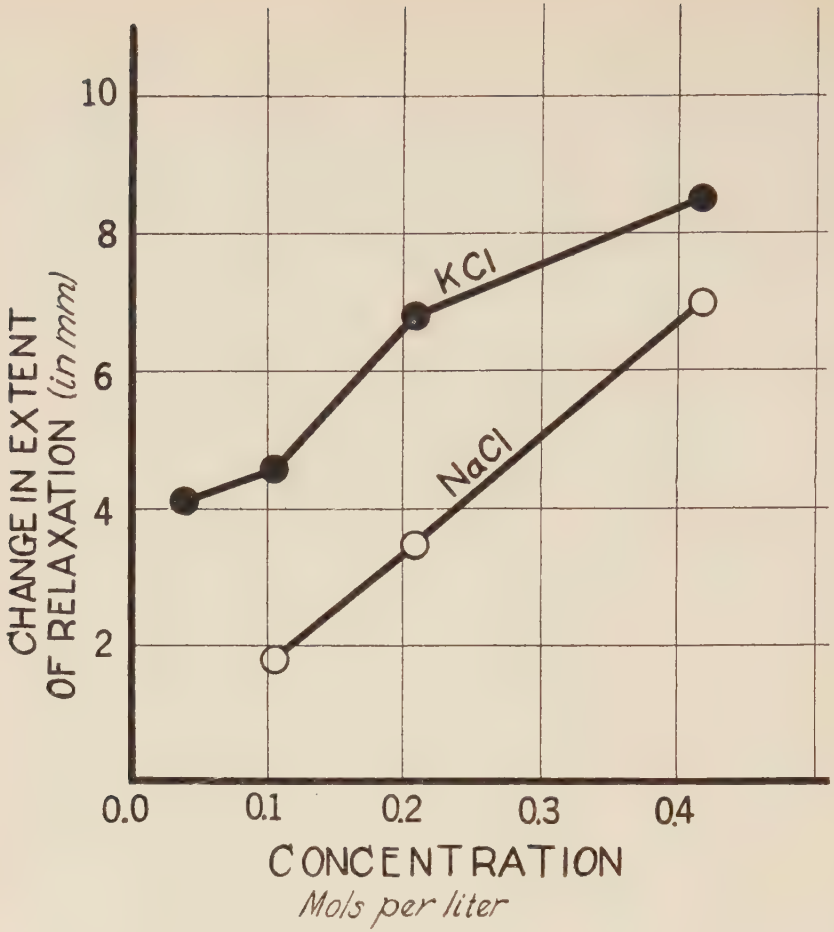
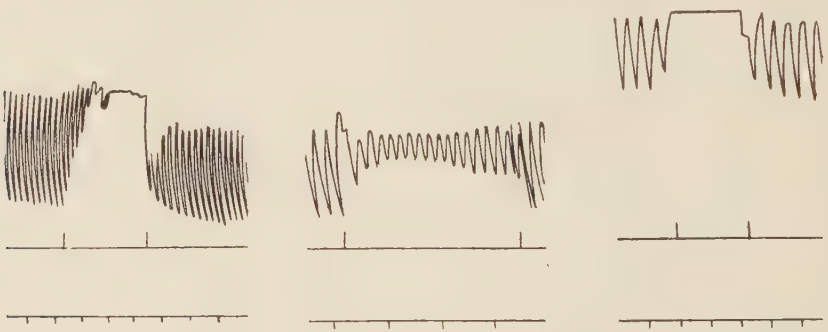


FIG. 5.—Graphs showing the relation between concentration of salt and the change in extent of relaxation. *Abscissas*, concentration of salt; *ordinates*, change in extent of relaxation.



A *B* *C*

FIG. 6.—Graphs showing the effect of excess and deficiency of KCl in van't Hoff solution. *A*, van't Hoff solution with KCl increased to 0.045 molar; *B*, van't Hoff solution with KCl decreased to 0.0045 molar; *C*, van't Hoff solution with no KCl.

At the second signal shown on graph *A* (Fig. 7) the heart was stimulated with an induction shock. The graph shows that this was followed by a single normal contraction. This demonstrates that the heart is still capable of contracting, indicating that the diastolic arrest was caused by interruption of automatic stimulation. Graph *A* also shows that when a heart, which has stopped in diastole from perfusion with a strong solution of $CaCl_2$, is again perfused with van't Hoff solution it immediately contracts very strongly, and then slowly relaxes until normal beating is resumed.

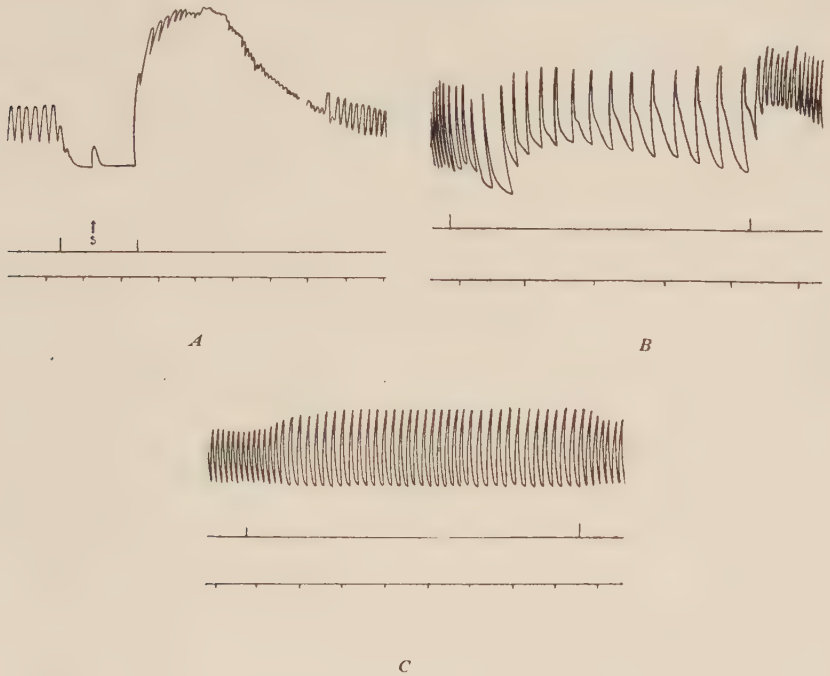


FIG. 7.—Graphs showing the effect of solutions of different concentrations of $CaCl_2$, made isotonic with the blood of the oyster by the addition of dextrose, and of van't Hoff solution with excess of $CaCl_2$. *A*, 0.418 molar; *B*, 0.104 molar; *C*, van't Hoff solution with $CaCl_2$ increased to 0.17 molar.

Table 3 shows that 0.209 molar $CaCl_2$ also causes the heart to stop immediately in diastole, but that the extent of relaxation and the extent of contraction preceding recovery on perfusion with van't Hoff solution are less than they are with 0.418 molar $CaCl_2$. Graph *B* (Fig. 7) and Table 3 show that with concentrations of $CaCl_2$ below 0.104 molar the heart continues beating, but with decrease in frequency; and that with decrease in concentration of $CaCl_2$ the frequency of beat increases and the amplitude of contraction decreases.

The foregoing results indicate that calcium acts in two ways on the heart of the oyster, i.e., that it depresses excitation and augments contraction. The decrease in frequency accompanying increase in concentration and the diastolic arrest which occurs in concentrated solutions are obviously due to inhibition of the mechanism associated with excitation rather than to an effect on the contraction itself, since under these conditions the heart still responds to artificial stimulation.

The increase in amplitude of contraction accompanying increase in concentration of $CaCl_2$ demonstrates the augmenting action of this salt on contraction, and it is believed that the abnormally strong contraction preceding recovery in van't Hoff solution of a heart stopped in diastole by means of perfusion with a strong solution of $CaCl_2$ also demonstrates this; i.e., the $CaCl_2$ that accumulates in the tissues during perfusion with the $CaCl_2$ solution causes augmentation of contraction on the resumption of automatic excitation in van't Hoff solution.

TABLE 3

EFFECT ON THE BEAT OF THE HEART OF THE OYSTER OF SOLUTIONS OF DIFFERENT CONCENTRATIONS OF $CaCl_2$, MADE ISOTONIC WITH THE BLOOD OF THE OYSTER BY THE ADDITION OF DEXTROSE, AND OF VAN'T HOFF SOLUTIONS WITH DIFFERENT DEGREES OF EXCESS AND DEFICIENCY OF $CaCl_2$

Concentration of $CaCl_2$	Percentage Change in Frequency	Percentage Change in Amplitude	Change in Extent of Relaxation (Mm.)
	$CaCl_2$ in Dextrose Solutions		
0.418 molar	−100	−100	+1.1
0.209	−100	−100	+0.6
0.104	− 71	+ 10	0.0
0.04	− 50	+ 3.6	0.0
	$CaCl_2$ in van't Hoff Solution (Excess)		
0.022	−15	+13	0.0
0.044	−25	+22	0.0
0.088	−25	+30	+0.1
0.17	−35	+48	+0.1
	$CaCl_2$ in van't Hoff Solution (Deficiency)		
0.003	+ 25	− 77	−3.0
0.005	+ 98	− 32	−0.8
0.008	+ 66	− 1.2	−0.2
0.0	−100	−100	0.0

EXCESS OF $CaCl_2$ IN VAN'T HOFF SOLUTION

Experiments were performed with van't Hoff solutions in which the concentration of $CaCl_2$ was increased, but without any change in the concentration of the other salts. Solutions in which the concentration of $CaCl_2$ was increased two, four, eight, and sixteen times (0.022 molar, 0.044 molar, 0.088 molar, and 0.17 molar, respectively) were used. The results for one experiment for one concentration are presented in graph *C* (Fig. 7), and the averages for all the concentrations used are presented in Table 3.

Graph *C* (Fig. 7) and Table 3 show that van't Hoff solutions containing excess of

$CaCl_2$ cause decrease in frequency of beat, increase in amplitude of contraction, and, in high concentration, increase in extent of relaxation. Figure 8 shows that the decrease in frequency and increase in amplitude of contraction vary directly with increase in $CaCl_2$ concentration.

These results obviously support the conclusion reached in the experiment on the effect of solutions of $CaCl_2$, namely, that calcium has a depressing effect on excitation as well as an augmenting effect on contraction.

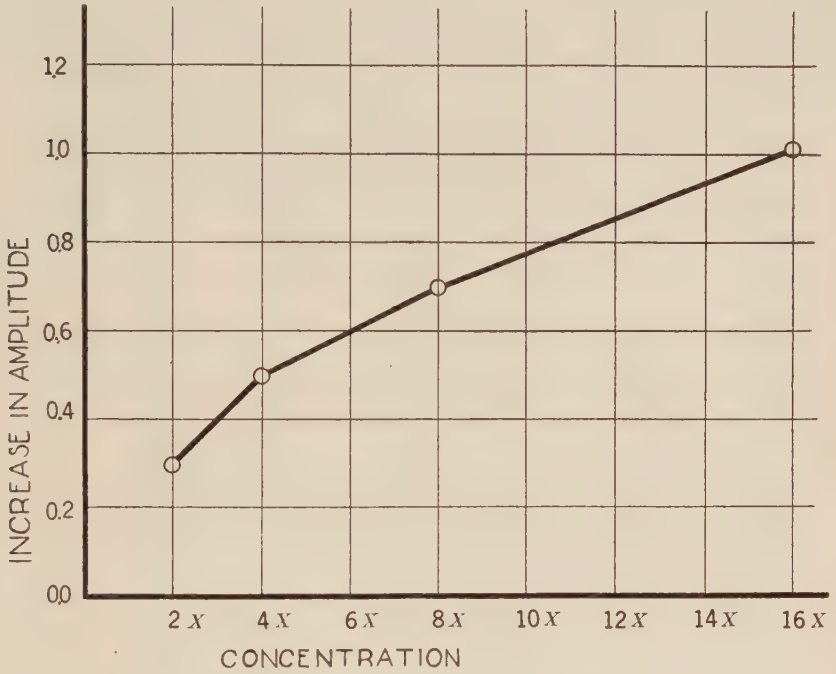


FIG. 8.—Graphs showing the relation between increase in concentration of $CaCl_2$ in van't Hoff solution and the change in amplitude of contraction. *Abscissas*, concentration of $CaCl_2$ (X, 0.011 molar $CaCl_2$, i.e., the concentration of $CaCl_2$ in van't Hoff solution); *ordinates*, change in amplitude of contraction.

DEFICIENCY OF $CaCl_2$ IN VAN'T HOFF SOLUTION

Experiments were performed in which the concentration of $CaCl_2$ in van't Hoff solution was decreased to 0.008 molar, 0.005 molar, 0.003 molar, and 0.0 molar, respectively. Figure 9*a* contains the results obtained in an experiment in which the heart was perfused successively with van't Hoff solutions containing 0.008 molar, 0.005 molar, and 0.003 molar $CaCl_2$, respectively. The results are essentially the same as those obtained in experiments in which each concentration was tested singly. Figure 9*b* shows the effect of complete absence of $CaCl_2$. Table 3 contains the averages of the results obtained with each of these solutions on a number of hearts.

Graph *A* (Fig. 9) and Table 3 show that perfusion with van't Hoff solution, with $CaCl_2$ decreased to 0.005 molar, causes inhibition of relaxation and increase in frequency, but does not affect the amplitude of contraction; that perfusion with van't Hoff solution

with the concentration of $CaCl_2$ lower than 0.005 molar causes decrease in extent of contraction as well as inhibition of relaxation and increase in frequency; and that perfusion with van't Hoff solution containing no $CaCl_2$ causes increase in length of the systolic period with gradual decrease in amplitude of contraction until the heart stops in diastole.

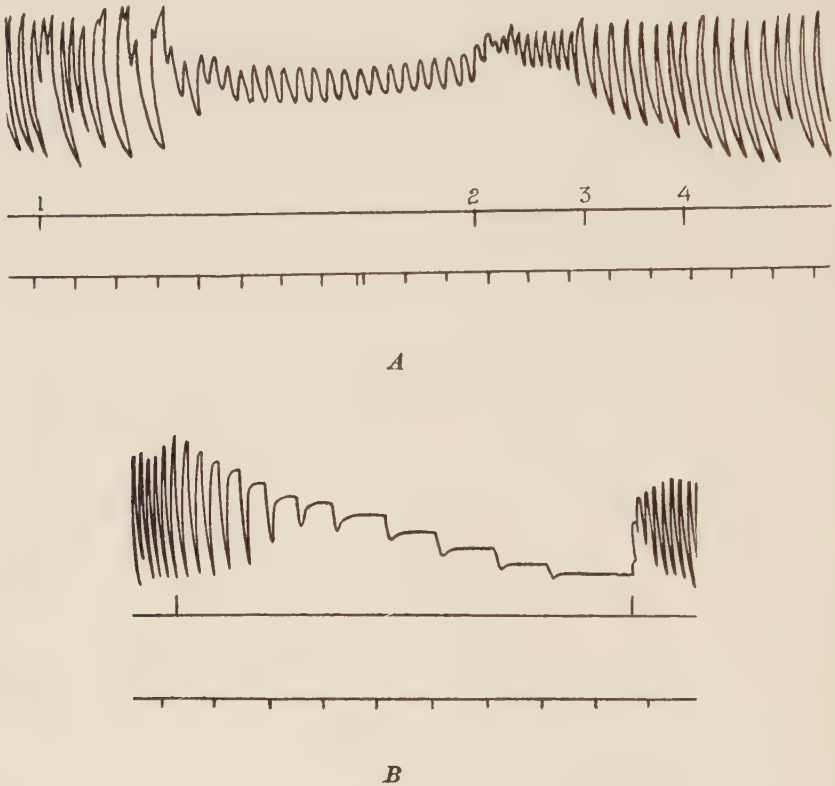


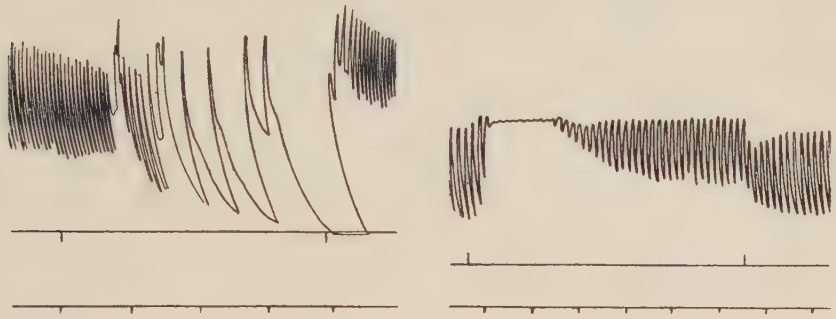
FIG. 9.—Graphs showing the effect of van't Hoff solution with deficiency of $CaCl_2$. *A:* (1) van't Hoff solution containing 0.011 molar $CaCl_2$ changed to van't Hoff solution containing 0.003 molar $CaCl_2$, (2) van't Hoff solution containing 0.003 molar $CaCl_2$ changed to 0.005 molar $CaCl_2$, (3) van't Hoff solution containing 0.005 molar $CaCl_2$ changed to van't Hoff solution containing 0.008 molar $CaCl_2$, (4) van't Hoff solution containing 0.008 molar $CaCl_2$ changed to van't Hoff solution containing 0.011 molar $CaCl_2$; *B*, effect of van't Hoff solution containing no $CaCl_2$.

It seems probable that the inhibition of relaxation and increase in frequency caused by van't Hoff solution with decreased concentration of $CaCl_2$ are due to diminished antagonism of the action of the other ions in van't Hoff solution. The decrease in the extent of contraction obtaining with $CaCl_2$ concentrations below 0.008 molar and the delayed diastolic arrest with complete lack of $CaCl_2$ indicate that, like vertebrate hearts, the oyster heart requires calcium for the functioning of the contractile mechanism.

MAGNESIUM CHLORIDE

PURE SOLUTIONS OF $MgCl_2$ IN DIFFERENT CONCENTRATIONS

Experiments were performed using two concentrations of $MgCl_2$ (0.418 molar and 0.209 molar). The results are presented in Figure 10 and Table 4. These show that solu-



A *B*

FIG. 10.—Graphs showing the effect of solutions containing different concentrations of $MgCl_2$, made isotonic with the blood of the oyster by the addition of dextrose, and of van't Hoff solution lacking magnesium salts. *A*, 0.418 molar $MgCl_2$; *B*, van't Hoff solution lacking magnesium salts.

TABLE 4

EFFECT ON THE BEAT OF THE HEART OF THE OYSTER OF SOLUTIONS OF DIFFERENT CONCENTRATIONS OF $MgCl_2$, MADE ISOTONIC WITH BLOOD OF THE OYSTER BY THE ADDITION OF DEXTROSE, AND OF VAN'T HOFF SOLUTION WITH DEFICIENCY OF MAGNESIUM SALTS

Concentration of $MgCl_2$	Percentage Change in Frequency	Percentage Change in Amplitude	Change in Extent of Relaxation (Mm.)
	$MgCl_2$ in Dextrose Solutions		
0.418 molar	−100	−100	+5.0
0.209	−100	−100	+4.5
	$MgCl_2$ in van't Hoff Solution (Deficiency)		
0.0	+20	−9.5	−0.3

tions of $MgCl_2$ in both these concentrations cause the heart to stop in a greatly relaxed state, but that the extent of relaxation is less with the lower concentration.

These results resemble those obtained with solutions of $CaCl_2$. The figure shows, however, that the abnormally strong contraction observed immediately after the

calcium solution was replaced by van't Hoff solution, did not occur when the magnesium solution was replaced by van't Hoff solution. The fact that contraction preceding recovery with van't Hoff solution did not occur indicates that magnesium does not have an augmenting effect on contraction like that of calcium.

DEFICIENCY OF MAGNESIUM IN VAN'T HOFF SOLUTION

Experiments were performed with solutions containing different concentrations of magnesium salts in van't Hoff solution. The concentration of the other ions was not changed. No definite changes were observed in the beat of the heart except with magnesium entirely lacking. The results obtained with van't Hoff solution without magnesium are presented in Figure 10 and Table 4. These results show that lack of magnesium in van't Hoff solution causes inhibition of relaxation and increase in frequency.

Comparison of the results concerning magnesium with those concerning calcium indicates that these ions have similar effects on excitation but that magnesium is not necessary for the functioning of the contractile mechanism, while calcium is. The increase in frequency and the inhibition of relaxation produced by lack of magnesium in van't Hoff solution indicate that magnesium, like calcium, antagonizes sodium and potassium.

DISCUSSION

The foregoing results show that the effects of NaCl, KCl, $CaCl_2$, and $MgCl_2$ on the heart of the oyster are not in complete accord with the effects of these salts on the hearts of vertebrates. The chief differences are that excess of KCl causes inhibition of relaxation in the heart of the oyster and inhibition of contraction in the hearts of vertebrates; and that large excess of $CaCl_2$ causes arrest in diastole in the former and arrest in systole in the latter. The question now arises as to how we may account for these differences.

Seliskar (1914) showed that the excitability of the ventricle of the turtle increases with decrease in concentration of $CaCl_2$. Ringer and others have found that excess of $CaCl_2$ causes decrease in frequency in the heart of the frog; and Ringer (1882), Locke and Rosenheim (1907), Mines (1912), and others have shown that calcium is necessary for contraction of the vertebrate heart and that excess of Ca causes increase in the force of contraction, resulting, in high concentration, in arrest in systole. From these results Clark (1927) has concluded that calcium acts specifically on each of two different systems in the heart, one associated with excitation, and the other with contraction; and that it depresses the former and augments the latter.

If calcium affects these two systems in the heart in different proportions in different animals, the fact that $CaCl_2$ in high concentrations causes arrest in diastole in the heart of the oyster and arrest in systole in the hearts of vertebrates is probably due to difference in the extent to which calcium affects excitation and contraction in the two forms; i.e., large excess probably produces in the heart of the oyster complete block of automatic excitation, resulting in immediate arrest in diastole, so that the effect of calcium on contraction remains latent until automatic excitation is resumed on return to van't Hoff solution, when excessive contraction obtains; and large excess probably produces in the hearts of vertebrates only slight depression of excitation, allowing the more marked effect on contraction to be manifested by arrest in systole.

Locke and Rosenheim (1907), Mines (1912), and others—by means of electric records taken during the diastolic arrest of the frog's heart induced by pure solutions of NaCl or KCl—have shown that the normal rhythm of excitation continues for some time in

these solutions, although the muscle fibers fail to respond; and they conclude that sodium and potassium have an inhibitory effect on contraction. Libbrecht (1920), Pond (1920), and others have shown that lack of NaCl or KCl stops automatic excitation without impairing the ability of the muscle to contract, and they maintain that these salts also have a stimulatory effect on excitation.

If these two salts affect excitation and contraction in different proportions in the hearts of different animals, the fact that excess of either salt causes in the oyster arrest in systole, and in vertebrates arrest in diastole, is probably referable to the difference in the degree of their influence on excitation and contraction in the two forms; i.e., excess probably produces in the heart of the oyster overstimulation of excitation without much inhibition of contraction, resulting in arrest in systole; while in the hearts of vertebrates the general effect is slight stimulation of excitation and great inhibition of contraction, resulting in diastolic arrest.

If both the depressing effect of calcium and the stimulating effect of sodium and potassium on excitation are exaggerated in the heart of the oyster, and similarly the effects of these elements on contraction are diminished, perfect antagonism between the effects of calcium and the effects of sodium plus potassium would obtain, these conditions being necessary for the proper functioning of the tissue. The foregoing results indicate that these conditions actually exist.

SUMMARY

1. The effects of NaCl, KCl, $CaCl_2$, and $MgCl_2$ were studied in the heart of the oyster by perfusing it with solutions of each salt in several concentrations, and with van't Hoff solution containing an excess or deficiency of each salt.

2. Solutions of NaCl and KCl inhibit relaxation and cause arrest in systole in high concentration. The extent of contraction at arrest varies directly with the concentration.

3. There is an optimum concentration of KCl in van't Hoff solution, and either increase or decrease in this concentration inhibits relaxation.

4. Solutions of $CaCl_2$ cause decrease in frequency of beat and increase in amplitude of contraction in low concentrations, and arrest in diastole in high concentrations. The decrease in frequency and the increase in amplitude of contraction vary directly with the concentration.

5. Excess of $CaCl_2$ in van't Hoff solution causes increase in amplitude and decrease in frequency, while deficiency of $CaCl_2$ in van't Hoff solution causes inhibition of relaxation and inhibition of contraction; and complete lack causes arrest in diastole.

6. Solutions of $MgCl_2$, like those of $CaCl_2$, produce arrest in diastole in high concentration, but they do not cause increase in amplitude of contraction as solutions of $CaCl_2$ do.

7. Complete lack of magnesium salts causes slight inhibition of relaxation.

8. An explanation is offered for the apparently opposite effects of solutions of NaCl, KCl, and $CaCl_2$ on the vertebrate heart and on the oyster heart.

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He received his preparatory education at Mt. St. Joseph's College and the Baltimore City College Evening High School. He spent two years at Mt. St. Mary's College, Emmitsburg, Md., after which he was admitted as a graduate student (New Plan) in the Department of Zoölogy, The Johns Hopkins University.

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